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MINIREVIEW

Influence of Neuronal Nicotinic Receptors over Nicotine Addiction and Withdrawal

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Cigarette smoking represents an enormous, global public health threat. Nearly five million premature deaths during a single year are attributable to smoking. Despite the resounding message of risks associated with smoking and numerous public health initiatives, cigarette smoking remains the most common preventable cause of disease in the United States. Fortunately, even in an adult smoker, smoking cessation can reverse many of the potential harmful effects. The symptoms associated with nicotine withdrawal represent the major obstacle to smoking cessation. This minireview examines the roles of various nicotinic receptors in the mechanisms of nicotine dependence, discusses the potential role of the habenula-interpeduncular nucleus axis in nicotine withdrawal, and highlights nicotinic receptors containing the $\beta 4$ subunit as a potential pharmacological target for smoking cessation strategies. Exp Biol Med 233:917–929, 2008

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Introduction

About 10 million cigarettes are sold every minute in the world and every eight seconds someone dies from tobacco use (1). If current trends continue, smoking will kill one in six people by 2030 (2). Cessation is the only effective

measure to prevent and limit the long-term negative effects of smoke (3).

Several phenomena participate in the initiation, maintenance and escalation of drug use that underlie nicotine dependence. The neuronal adaptations produced by repeated exposure to nicotine include a greater expression of neuronal nicotinic acetylcholine receptors (nAChRs), the need of progressively higher doses of nicotine to obtain the same effect (tolerance), and the increased ability of the drug to activate dopaminergic neurotransmission and trigger appetitive behaviors (sensitization) (4, 5). Most smokers recognize the negative impact of smoking on health and would prefer to quit, if possible. However, very few actually succeed (6). This happens mainly because of the withdrawal symptoms that appear upon smoking cessation. In fact, withdrawal symptoms are a better predictor of unsuccessful quit attempts than smoke intake or dependence (7). While current therapies for smoking cessation are helpful, none can claim a very high rate of success (8). Therefore, there is a great need to better understand the interacting behavioral and biological factors that lead to the physical and psychological manifestations of nicotine dependence.

This brief review examines the roles of each nAChR subunit in nicotine addiction, with special emphasis on nicotine withdrawal. In addition, we present the view that nAChRs in the medial habenula (MHb) and interpeduncular nucleus (IPN) have great influence on the symptoms of nicotine abstinence and represent a novel target for smoking cessation therapies.

Nicotinic Acetylcholine Receptors Bind the Nicotine Contained in Tobacco

Neuronal nAChRs are pentameric ligand gated ion channels formed by either α subunits ($\alpha 7$, $\alpha 9$, $\alpha 10$) or combinations of α and β subunits ($\alpha 2$ - $\alpha 6$ and $\beta 2$ - $\beta 4$) (9). They bind the acetylcholine (ACh) released mainly by neurons located in the pedunculopontine tegmentum and the

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laterodorsal pontine tegmentum, several basal forebrain nuclei, or the striatum (9). Upon opening, nAChRs are permeable to monovalent and divalent cations, mainly Na^+ and Ca^{2+} (9). The membrane depolarization that follows ion permeation can trigger a variety of intracellular events, including the activation of signal transduction cascades and the transcription of genes (10). Besides undergoing transitions between the open and closed states nAChRs can also exist in the desensitized state (11). The kinetics of each conformational state are influenced by the subunit composition of the channel (12) and might be relevant for the mechanisms of nicotine dependence (13).

Several studies on mRNA and protein expression levels have shown that different areas of the brain express specific subsets of nAChR subunits. This anatomical knowledge, together with studies in genetically modified mice, has shed light over the possible functions of several nAChR subunits. Experimental evidence from several groups has confirmed the role of $\alpha 4\beta 2$ nAChRs in addiction (14, 15). Those receptors are necessary and sufficient for nicotine reward, tolerance and sensitization (16). Another subunit that may play a key role in nicotine addiction is $\alpha 6$, because $\alpha 6$ -containing receptors seem to dominate nicotine control of dopamine neurotransmission in the nucleus accumbens (17). Interestingly, we showed that $\beta 4$ -containing ($\beta 4^*$) (19), but not $\beta 2$ -containing ($\beta 2^*$) nAChRs (18, 19) are necessary for the expression of the somatic signs of nicotine withdrawal. More recent data indicate that $\alpha 7^*$ nAChRs can also influence the somatic signs of withdrawal (20). Table 1 summarizes the main expression patterns and functions of each nAChR subunit.

Withdrawal Symptoms in Humans

In humans, cessation of tobacco intake precipitates both somatic and affective symptoms of withdrawal which may include severe craving for nicotine, irritability, anxiety, loss of concentration, restlessness, decreased heart rate, depressed mood, impatience, insomnia, and increased appetite and weight gain (21, 22). These withdrawal symptoms cause enough distress to become a deterrent to abstinence and a drive to relapse (7). The negative affective symptoms usually start 4 to 24 h after the last cigarette, peak in about three days and may not recede even after a month of tobacco abstinence (8, 21, 23–27).

Anxiety and stress have a complex influence on all aspects of nicotine dependence, including the withdrawal syndrome. While many smokers use cigarettes as a tool to attenuate stress and anxiety and maintain that smoking has a calming effect (28–30), anxiety increases during withdrawal. Indeed, several studies suggest that smokers continue to smoke to avoid that particular symptom of nicotine deprivation (31–38). Besides being a product of nicotine withdrawal, stress and anxiety have the ability to exacerbate its symptoms, which results in increased craving and relapse (36, 39–46).

Similarly to anxiety, depression may both promote smoking and be a symptom of nicotine withdrawal (47–49). Subjects with a history of depression seem more sensitive to the effects of nicotine and display elevated craving and withdrawal scores upon nicotine cessation (50). Finally, another stress-related disorder, PTSD (post-traumatic stress disorder) is also associated with increased smoking behavior and enhanced withdrawal symptoms (51, 52), purporting mood regulation as one of the factors prompting smoking and preventing smoking cessation.

Withdrawal Symptoms in Rodents

Withdrawal symptoms can be assessed in animals by the sudden discontinuation of chronic nicotine administration and recording of withdrawal signs, either through observation of behavioral signs or through monitoring of disruptions in operant behavior. Alternatively, withdrawal can be precipitated on chronic nicotine treated rodents by systemically administering nAChR antagonists. Nicotine withdrawal signs are both physical, or somatic, and affective, or non-somatic.

Somatic Signs. Somatic signs of withdrawal upon interruption of chronic nicotine treatment were first documented in the rat (53). These signs included teeth chattering, chews, gasps, palpebral ptosis, tremors, shakes, and yawns. The number of signs depended on the amount of nicotine infused, and were relieved by acute nicotine treatment (53). Another way of precipitating withdrawal in rats chronically treated with nicotine is the systemic injection of nicotinic antagonists such as mecamylamine, di-hydro-beta-erythroidine, or methyllycaconitine (54). Mecamylamine, which has a slightly higher affinity for $\alpha 3\beta 4^*$ nAChRs than for $\beta 2^*$ receptors, is the best antagonist at precipitating nicotine withdrawal (54).

The advent of genetic engineering techniques has prompted the study of nicotine withdrawal symptoms in the mouse. The symptoms of nicotine withdrawal are qualitatively similar to those observed in rats and consist of a sharp increase in certain normal behaviors that become repetitive and more frequent. Among those behaviors there are shaking, grooming and scratching. In addition, behaviors such as jumping, which are not normally observed, also appear in the mouse undergoing nicotine withdrawal (54, 55). Our lab has shown that mice null for the $\beta 4$ subunit show no somatic signs when nicotine withdrawal is precipitated with systemic mecamylamine, and no spontaneous withdrawal-induced hyperalgesia (19). In the same report we also showed that $\beta 2^{-/-}$ mice show normal mecamylamine-precipitated somatic signs of behavior (19), a finding that was later confirmed by an independent group (18). In addition, we have shown that the $\alpha 7$ subunit also plays a role in mecamylamine-precipitated somatic signs of withdrawal. We showed that $\alpha 7^{-/-}$ mice show an intermediate withdrawal phenotype on that experiment (20). Interestingly, it was reported by another group that

Table 1. nAChR Subunits, Their RNA Expression Patterns and the Major Phenotypes of Mutant Mice for Each Subunit^a

Subunit	Expression	Major mutant mouse phenotypes	References
$\alpha 2$	High: IPN, amygdala Low: olf, basal ganglia, hippo, cx	$\alpha 2$ -/- mice show impaired hippocampal LTP, suggesting an effect on learning and memory.	Ishii et al., 2005 (193); Nakauchi et al., 2007 (194)
$\alpha 3$	High: olf, MHb, IPN, pineal, #10 Low: thal, hypothal, SN/VTA, Sup col, cx	$\alpha 3$ -/- mice die days after birth. $\alpha 3$ +/- mice show decreased nicotine-induced seizures.	Salas et al., 2004a (19); Xu et al., 1999 (195)
$\alpha 4$	High: cx, VTA, striatum, thal, hypothal, MHb, etc.	$\alpha 4$ -/- mice show altered nicotine-induced DA levels in the striatum. Activation of receptors in $\alpha 4$ gain of function mice is sufficient for reward, tolerance and sensitization.	Marubio et al., 2003 (196); Tapper et al., 2004 (16)
$\alpha 5$	High: hippo (CA1), IPN, SN/VTA, #10 Low: Cx	Decreased nicotine-induced hypolocomotion and seizures. Impaired autonomic function under certain conditions.	Salas et al., 2003 (185); Wang et al., 2002 (197)
$\alpha 6$	High: SN/VTA	$\alpha 6$ is a partner of the $\beta 3$ and $\beta 2$ subunits. It dominates the nicotine control of dopamine neurotransmission in nucleus accumbens.	Champtiaux et al., 2002 (198); Exley et al., 2007 (17)
$\alpha 7$	High: cx, hippo, thal, VTA, striatum, etc.	No major behavioral phenotype. Normal sensitivity to acute nicotine. Decreased nicotine withdrawal somatic signs and hypernociception. $\alpha 7$ gain of function mice show increased sensitivity to nicotine-induced seizures.	Broide et al., 2002 (199); Franceschini et al., 2002 (200); Grabus et al., 2005 (56); Paylor et al., 1998 (201); Salas et al., 2007 (20)
$\alpha 9/\alpha 10$	High: vestibular and cochlear hair cells	Related to hearing function. No known involvement with nicotine addiction.	Elgoyhen et al., 1994 (202); Elgoyhen et al., 2001 (203)
$\beta 2$	High: cx, VTA, striatum, thal, hypothal, MHb, etc.	Implicated in nicotine reinforcement and self-administration. These phenotypes were rescued by viral re-expression of $\beta 2$ in the VTA of $\beta 2$ -/- mice. $\beta 2$ -/- mice show normal somatic signs of nicotine withdrawal.	Maskos et al., 2005 (14); Picciotto et al., 1998 (15); Salas et al., 2004b (186)
$\beta 3$	High: MHb, SN/VTA Low: Sup Col, LC	The α -conotoxin MII-sensitive component of nicotine-induced DA release is lost in $\beta 3$ -/- mice, which also show less anxiety-like behavior.	Booker et al., 2007 (204); Cui et al., 2003 (205)
$\beta 4$	High: olf, MHb, IPN, pineal, #10	$\beta 4$ -/- mice show decreased anxiety-like behavior, are insensitive to nicotine-induced seizures and hypolocomotion. Do not show somatic signs of nicotine withdrawal.	Salas et al., 2004a (19); Salas et al., 2004b (186); Salas et al., 2003 (206)

^a IPN, interpeduncular nucleus; olf, olfactory bulb; hippo, hippocampus; cx, cortex; MHb, medial habenula; #10, cranial nerve #10; thal, thalamus; hypothal, hypothalamus; SN/VTA substantia nigra/ventral tegmental area; Sup Col, superior colliculus; LC, locus coeruleus.

$\alpha 7$ -/- mice show normal somatic signs of spontaneous withdrawal, but decreased hyperalgesia upon spontaneous nicotine withdrawal (56). The apparent differences between these two reports may be due to the different protocols followed.

Non-Somatic Signs. There are four main measures of affective signs of withdrawal that have consistently been reported on rodents: anhedonia, conditioned place aversion, anxiety-related behavior and conditioned fear.

A major affective symptom of nicotine withdrawal is a diminished interest in rewarding stimuli, or anhedonia (57). Interestingly, depression, which is highly correlated to nicotine withdrawal, also produces anhedonia (58). In rats, anhedonia is measured as an increase in brain-stimulation reward thresholds (57). Withdrawal from several drugs of

abuse, including nicotine, significantly elevates brain-stimulation reward thresholds, reflecting lower interest in the electrical stimuli that seem rewarding in basal conditions (58). Both spontaneous (59, 60) and mecamylamine-precipitated (61) nicotine withdrawal induce increases in self-stimulation thresholds.

Conditioned place aversion is another paradigm used to reveal the affective signs of withdrawal (58). After exposure to a two-chamber apparatus, chronic nicotine-treated animals are injected with an antagonist to precipitate withdrawal, and immediately confined in one of the chambers. Following injection of saline, animals are confined in the other compartment. When tested without drug and with access to both chambers, animals prefer to stay in the chamber that has been paired with saline and not

in the chamber paired with antagonist and therefore, withdrawal symptoms. Different antagonists and rat strains have been used in this paradigm, revealing an effect of both strain and type of drug on the amount of antagonist needed to observe place aversion (61, 62).

The third non-somatic manifestation of nicotine withdrawal is increased anxiety-like behavior in the elevated plus maze (EPM). In the EPM the animal explores 4 corridors arranged in a plus sign shape. Two of the corridors have tall walls while the other 2 are without walls, and the maze is elevated 50 cm from the floor. Rodent behavior on this maze has been repeatedly used as a model for anxiety (63). Mice and rats undergoing nicotine withdrawal showed increased anxiety-like behavior in the EPM (54, 64). This phenomenon mirrors the increase in anxiety reported by humans experiencing nicotine abstinence and suggests that the sensitivity to anxiety might influence the degree of withdrawal signs.

Cognitive symptoms of withdrawal can be explored in rodents with the conditioned fear paradigm (65). In this task, animals are trained in a context by pairing an auditory conditioned stimulus (CS) with a foot shock unconditioned stimulus (US). The association formed between the training context and the US (contextual fear conditioning) requires the hippocampus, while the association between the CS and the US (cued fear conditioning) does not require the hippocampus (66, 67). Nicotine withdrawal produces deficits in contextual fear conditioning and seems to selectively affect the acquisition of but not the recall or expression of the learned response (68). $\beta 2^*$ nAChR are involved in this aspect of the withdrawal syndrome (68).

Current Pharmacotherapies for Smoking Cessation

Nicotine Replacement Therapy. Nicotine replacement therapy (NRT) is usually the first choice for those smokers that want to quit. NRT provides an alternate source of nicotine without the tars and poisonous gases found in cigarettes. It promotes smoking cessation by allowing smokers to control cravings while they gradually decrease nicotine intake. NRT is available as transdermal patches, chewing gum, nasal sprays, inhalers, sublingual tablets, and lozenges. Except for the nasal spray, all other forms of NRT deliver nicotine more slowly than cigarettes. NRT is effective at reducing craving and withdrawal associated with quitting (69). However, given the rapid rise in nicotine levels during smoking, NRT users may still be able to obtain additional reinforcement from cigarettes during treatment (70). This phenomenon, coupled with the sensory cues that further maintain tobacco dependence (71) make the success rate of NRT much lower than desirable.

Partial Agonists. A second approach to smoking cessation is the treatment with a combination of nicotine and a non-selective nAChR antagonist to achieve partial receptor agonism. Mechanistically, this combination of

agents is expected to target the nAChRs that mediate the reinforcing effects of nicotine. Due to the presence of the antagonist, the effects of nicotine are attenuated so that its reinforcing effects are diminished but are still sufficient to prevent craving. The administration of the non-selective nAChR antagonist mecamylamine alone was one of the earliest suggestions for smoking cessation pharmacotherapy (72). Mecamylamine dose-dependently reduced the subjective effects of nicotine in some smokers (73). The results, however, were inconsistent in that mecamylamine increased cigarette consumption in some studies. In addition, side effects compromised compliance. Rose and Levin (74) were the first to propose the co-administration of nicotine and mecamylamine. Small clinical trials using transdermal nicotine and oral mecamylamine showed that this approach might be valuable, as the combination achieved higher abstinence rates than did transdermal nicotine alone (75). Despite the potential benefits of this pharmacological strategy, administering two separate compounds with different pharmacokinetic and metabolic profiles under the condition of maintaining a narrow agonist-to-antagonist ratio can be extremely challenging. Nevertheless, this attempt introduced the concept of partial nAChR agonism, especially at $\alpha 4\beta 2^*$ nAChRs, as a strategy for smoking cessation. The use of cytisine further supported the concept. Cytisine, a nAChR partial agonist (76), is a plant alkaloid that has been used for over 40 years in Eastern Europe, but not in the US, as a smoking cessation agent (77).

Varenicline, the first partial nicotinic agonist approved in the USA as a therapeutic aid to smoking cessation, was developed by merging structural elements of nicotinic and opioid ligands (78, 79). The rationale behind varenicline's clinical efficacy is the one previously discussed. When nicotine is not present it acts as a partial agonist, providing at least part of the reinforcing effects of nicotine a smoker is used to receiving through nicotine. This effect would reduce cravings and withdrawal when nicotine is not present. In contrast, when nicotine is present, varenicline would act as an antagonist, preventing the rewarding effects of nicotine (80). In fact, varenicline has effects on dopamine release that are similar to those of nicotine, but at a smaller scale (80). In a study comparing varenicline with bupropion (brand name Zyban, see below) and placebo, 23% of smokers taking varenicline, 15% taking bupropion and 10% taking placebo were able to quit and remained abstinent for one year (81). It should be noted that although varenicline was conceived and marketed as a $\alpha 4/\beta 2$ -specific partial agonist, it is also a full agonist at $\alpha 7^*$, and a partial agonist at $\alpha 3\beta 4^*$ nAChRs. In fact, the efficacy of varenicline as a partial agonist is much higher for $\alpha 3\beta 4^*$ and $\alpha 7^*$ nAChRs than it is for $\alpha 4\beta 2^*$ receptors (82). Therefore, it is possible that nAChR subtypes other than those containing $\alpha 4$ and $\beta 2$ are also implicated in the effects of varenicline on nicotine withdrawal.

Bupropion. Bupropion is an atypical antidepressant (83) marketed as Wellbutrin for its antidepressing effects,

and as Zyban (Glaxo Wellcome) for its anti-tobacco properties. It was the first non-nicotine-based therapy approved by the Food and Drug Administration (FDA) for smoking cessation (84). The mechanisms of bupropion's effects on depression and tobacco smoke seem independent from one another, i.e. the efficacy as anti-tobacco drug does not depend on the presence of current or past history of depression (85). Bupropion can double long-term abstinence rates compared to placebo (21) by decreasing both cue-induced tobacco craving (86) and withdrawal symptoms such as depression, difficulty concentrating and irritability (87).

Animal studies have shown that bupropion alters brain reward circuits influenced by nicotine, reversing the elevated intracranial self-stimulation thresholds resulting from nicotine abstinence (88), thus impairing the negative reinforcing effects of nicotine (59). In rodents, bupropion can alter nicotine reinforcement with some doses causing a decrease in nicotine self-administration behavior (89–91). However, a biphasic dose-response pattern to nicotine has also been reported, with low doses of bupropion increasing nicotine infusions and high doses decreasing responding non-specifically (89, 91). In addition, bupropion has been shown to attenuate the nicotine abstinence syndrome in rats. Acute and chronic bupropion exposures alleviate the expression of somatic signs associated with spontaneous and precipitated withdrawal, respectively, and chronic exposure also reduces the place aversion conditioned to mecamylamine-precipitated nicotine abstinence (88, 92).

Although the mechanisms of action of bupropion on nicotine addiction remain uncertain, bupropion is known to affect several systems involved in addiction. First, bupropion decreases DA reuptake in the mesolimbic system (93). Bupropion also inhibits noradrenaline (NE) reuptake in the locus coeruleus (93, 94), which is thought to be involved in nicotine withdrawal (95). Finally, bupropion also acts as an antagonist for nAChRs, including the $\alpha 3\beta 4^*$ subtype (96–98).

Cannabinoid Receptor Antagonists. Central cannabinoid (CB) receptors, especially the CB1 subtype, have been recently implicated in brain reward function due to the ability of endocannabinoids to increase dopamine (DA) levels in the mesolimbic system (99–101). Interestingly, $\Delta 9$ tetrahydrocannabinol (THC), a CB receptor antagonist, potentiates the effects of non-pharmacologically active doses of nicotine (102), suggesting an interaction between the nicotinic and the cannabinoid systems. Therefore, CB1 receptor antagonists represent potential aids for smoking cessation. Indeed, the CB1 receptor antagonist rimonabant (SR 141716, trade name Acomplia) has been shown to reduce the motivational effects of nicotine in the conditioned place preference and the nicotine self-administration paradigms (103, 104). Rimonabant was the first CB1 receptor antagonist to be clinically tested in the European Union and it is currently under Phase III clinical trials in the US. The drug was initially developed as a possible treatment

for obesity as CB1 receptors participate in the control of food consumption and energy expenditure. It has also been proposed as a smoking cessation aid and may protect successful quitters from significant post-cessation weight gain (105, 106). The beneficial effects of rimonabant on weight loss have been demonstrated in a recent Cochrane review (107). Since weight gain is one reason why some smokers avoid quit attempts, the dual action of rimonabant on decreasing weight gain and on smoking cessation may be particularly useful, especially when cardiac risk factors (obesity and tobacco smoke being two critical factors) are taken into account (108).

Mechanisms of Nicotine Withdrawal

nAChRs are expressed throughout the CNS and can influence a number of brain areas and functions. The nicotine contained in tobacco produces neuroadaptations that may explain the alteration in brain reward systems involved in the addiction process (109). Such neuroadaptations reflect nicotine's influences on several neurotransmitter systems, including acetylcholine, dopamine (DA), opioid peptides, serotonin (5-HT), and glutamate. Abrupt cessation of nicotine is likely to alter the neurochemistry of the addicted brain, thus triggering the affective and somatic signs of withdrawal. Neuroadaptations that affect multiple neurotransmitter systems are common to other drugs of abuse. One example is given by opiate addiction in which a number of non-opioid transmitters have been postulated to be involved in the development of both dependence and abstinence (110–113). The symptoms of nicotine abstinence may therefore reflect the activation of several brain circuits. How each brain circuit is altered during both nicotine exposure and withdrawal is likely to depend on the pharmacological and biophysical properties of the nAChR subtypes expressed in the brain areas that are important for that circuit.

Dopamine and Nicotine Withdrawal. The mesolimbic dopaminergic system serves a fundamental role in the acquisition of behaviors that are inappropriately reinforced by addictive drugs, and is very likely to also participate in the mechanisms of withdrawal (114). In the nucleus accumbens (NAcc), spontaneous or mecamylamine-precipitated nicotine withdrawal is associated with a decrease in extracellular DA levels (115–117). Whether altered DA levels participate in both the somatic and motivational aversive aspects of withdrawal is not clear (58, 118). In fact, the somatic signs of withdrawal seem to appear earlier than the decreases in accumbal DA output (115). This temporal dissociation between the two phenomena suggests that accumbal DA may not necessarily be involved in mediating the somatic aspects of nicotine withdrawal. Interestingly, the opioid receptor antagonist naloxone can increase somatic withdrawal signs in nicotine-dependent rats without affecting accumbal DA release (119).

In addition to the NAcc, DA fibers that arise within the

ventral tegmental area (VTA) also project to the prefrontal cortex (PFC). In contrast to the deficits in DA transmission observed in the NAcc, nicotine withdrawal increases DA output in the PFC of rats (119). Such increases in PFC DA release may be important in mediating aversive aspects of nicotine withdrawal, as enhanced DA transmission in the PFC has been observed during exposure to stressful and aversive stimuli (120–122), and has been implicated in mediating anxiety-related behaviors (123, 124). As previously discussed, anxiety is one of the manifestations of nicotine withdrawal in humans as well as in animal models of addiction.

Norepinephrine and Nicotine Withdrawal. Nicotine enhances the release of norepinephrine (NE) in various CNS regions (125, 126), and NE mechanisms can modulate midbrain DA function (127). In addition, the NE reuptake inhibitor reboxetine attenuates nicotine self-administration (128), and bupropion, which has an NE component to its action, is used in smoking cessation treatments (129). The noradrenergic tricyclic antidepressant nortriptyline, which inhibits serotonin and noradrenaline reuptake, could also work as second-line therapy for smoking cessation (130). Despite this evidence suggesting that noradrenergic mechanisms might be important for nicotine abuse, not much is known on the role of NE in the mechanisms of nicotine withdrawal. One report indicates that nicotine withdrawal alters NE levels in the hypothalamus and cortex of mice exposed to chronic nicotine in the drinking water (131). $\alpha 7^*$ nAChRs might be involved in this phenomenon (132). The paucity of data on nicotine contrasts with the abundant literature implicating noradrenergic mechanisms in opioid withdrawal. For example, withdrawal from chronic morphine increases NE release in the cortex and the bed nucleus of the stria terminalis (BNST) (133–135). NE release in the BNST may underlie anxiety associated with protracted withdrawal.

Serotonin and Nicotine Withdrawal. 5-HT is also expected to influence nicotine reward, as nicotine increases 5-HT release in the cortex, striatum, hippocampus, dorsal raphe nucleus, hypothalamus, and spinal cord (136, 137). In addition, DA neurons are influenced by 5-HT mechanisms (138). As for the role of 5-HT in withdrawal, it has been proposed that reduced serotonergic neurotransmission may contribute to the anhedonia observed during both amphetamine and nicotine withdrawal in humans (60, 139, 140). The role of the various 5-HT receptors and the anatomical localization of the 5-HT/withdrawal interaction are less clear. Some investigators suggested that during nicotine withdrawal, 5-HT activates inhibitory somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei leading to a decrease in 5-HT release into forebrain and limbic sites (141, 142). This conclusion is supported by the observation that a serotonergic antidepressant treatment that combines the 5-HT-selective re-uptake inhibitor fluoxetine and a 5-HT_{1A} receptor antagonist rapidly reverses the elevation in brain-stimulation reward thresholds observed in rats undergoing

nicotine withdrawal (60, 143). Contrary to the view that reduced serotonergic transmission contributes to nicotine withdrawal, Cheeta and colleagues showed that administration of nicotine directly into the dorsal raphe nucleus, at a concentration that activates somatodendritic 5-HT_{1A} receptors, reverses the increase in anxiety observed in rats undergoing nicotine withdrawal as measured in the social interaction test (144). Other studies have implicated the activation of 5-HT₃ receptors in the amygdala in the heightened anxiety observed during nicotine withdrawal (145).

Opioids and Nicotine Withdrawal. The first model for nicotine withdrawal in rodents was developed by modification of a previous model used in opioid research (53). The behavioral similarities between these two models prompted a follow-up study, where rats treated chronically with nicotine were injected with the opioid receptor antagonist, naloxone, and nicotine withdrawal symptoms became apparent (146). In a separate experiment, acute morphine injection diminished spontaneous nicotine withdrawal, consistent with a major role for the opioid system in nicotine withdrawal (146). Genetic approaches have also demonstrated the impact of the opioid system in nicotine withdrawal in mice. A role for endogenous enkephalins on nicotine withdrawal was investigated by using preproenkephalin knock-out mice (147). In these mice the somatic expression of mecamylamine-precipitated nicotine withdrawal was significantly attenuated. Other effects of nicotine such as nicotine-induced antinociception, conditioned place preference, and enhancement in DA extracellular levels in the nucleus accumbens induced by nicotine were also reduced in preproenkephalin-deficient mice, demonstrating an important role for the endogenous opioid system not only in nicotine withdrawal but also in nicotine rewarding properties (147). Another interesting observation is that 18-Methoxycoronaridine (18-MC), a potent antagonist of $\alpha 3\beta 4$ nicotinic receptors, inhibits systemic morphine-induced increases in DA levels when injected into the rat habenula or IPN (148).

Glutamate and Nicotine Withdrawal. Nicotine, acting at presynaptic receptors, enhances glutamate release in several areas of the brain including the VTA, PFC and NAcc (149, 150). The glutamate released upon nicotine exposure binds to metabotropic and ionotropic glutamate receptors on postsynaptic dopaminergic neurons thereby increasing their bursting activity and increasing dopamine release. These actions may partly mediate the reinforcing effects of acute nicotine (150) as antagonists of the mGluR5 receptor subtype have been shown to decrease both intravenous nicotine self-administration (151) and cue-induced reinstatement of nicotine seeking (152). Other metabotropic glutamate receptor subtypes may be involved in the nicotine withdrawal syndrome, in particular in the negative affective symptoms. As previously discussed, nicotine withdrawal produces anhedonia, or diminished interest in pleasure. In rodents, anhedonia is believed to

produce an increase in the threshold for stimulation in the intracranial self-stimulation procedure (60). This elevation in threshold is blocked by mGluR2/3 antagonists (153). Therefore, mGluR5 affects nicotine self-administration while mGluR2/3 affects nicotine withdrawal. This parallels the roles of the $\beta 2$ and $\beta 4$ nAChR subunits in rodents. Interestingly, mGluR5 is expressed in several areas of the brain, but not in the MHb, while mGluR3 is expressed in a more restricted pattern, with high levels in the MHb, among other areas (154).

Monoamino Oxidase Inhibitors and Nicotine Withdrawal. Although it is known that nicotine is the main addictive component of tobacco, tobacco smoke contains many other compounds including monoamino oxidase inhibitors (MAOIs). It has been shown that whereas behavioral sensitization to D-amphetamine stayed constant following up to 30 days of withdrawal, similar conditions abolished behavioral sensitization to nicotine. Following 30 days of withdrawal, locomotor responses to nicotine were identical to those in naïve mice. However, when the MAOIs tranylcypromine or pargyline were co-injected with nicotine, behavioral sensitization was maintained even after long-term withdrawal (155). In a similar report, it was shown that in nicotine-infused rats, mecamylamine induced a place aversion that lasted 6 weeks. When nicotine-infused rats were also treated with a MAOI, mecamylamine-induced conditioned place aversion persisted for at least 8 months of abstinence. In addition, the MAOI treatment slightly decreased ratings of somatic signs induced by mecamylamine administration (156). These data suggest that nicotine may not be the only psychoactive substance in tobacco, and that MAOIs may potentiate the effects of nicotine.

Brain Regions Associated to Drug Withdrawal. A widely accepted view of the mechanisms of drug addiction is that the reinforcing effects of most drugs of abuse depend on induction of increases in DA levels in the nucleus accumbens. DA has been implicated in motor and cognitive function, and in regulation of reward, saliency and motivation (157–159). The NAcc receives dopaminergic innervation from the VTA, and it has been shown that $\beta 2^*$ nAChRs in the VTA are necessary for the rewarding effects of nicotine (14). The PFC, which has connections to both NAcc and VTA, is usually considered as the third region in this circuit (157). Other areas of the brain have also been shown to be important for the effect of drugs of abuse, such as the amygdala (160) and the hypothalamus (160). The involvement of the VTA/NAcc/PFC in addiction has been extensively reviewed elsewhere (157, 159). We will focus on the role of the habenula and interpeduncular nucleus on nicotine withdrawal.

The Role of the Habenula in Drug Withdrawal. The role of DA in reward has been best defined in the concept of ‘incentive salience’, in which DA levels are involved in reward prediction for the purpose of reward seeking (161). In monkeys, dopaminergic cells were shown to fire in response to reward. After training with a cue that

predicts reward, dopaminergic cells fired in response to the prediction of reward, and not to the reward itself. Interestingly, dopaminergic cells fired less than normal if reward was not delivered when expected (162). A recent report (163), as well as a body of literature from the 1980s (164–166) points to the habenula as the region that controls the decrease in firing of dopaminergic cells in the NAcc.

The habenula has been implicated in withdrawal to drugs of addiction by the use of ibogaine and its derivative, 18-Methoxycoronaridine (MC-18). Ibogaine is an alkaloid extracted from the African shrub *Tabemanthe Iboga* (167). Several chemical addictions such as opiate, cocaine, nicotine and alcohol might be sensitive to the effects of ibogaine (168). The effects of ibogaine in rats include a decrease in self-administration, a decrease in withdrawal signs and a block of drug induced DA release. Ibogaine’s effects might reflect the interaction with multiple neurotransmitter systems as the alkaloid acts at several neurotransmitter receptors such as sigma opioid, NMDA and nAChRs (169). More recently, a derivative of ibogaine, 18-Methoxycoronaridine (18-MC), was synthesized and used in similar experiments (170). In rodents, 18-MC decreased morphine, cocaine, methamphetamine, alcohol and nicotine self-administration, and opioid withdrawal signs. 18-MC also blocked the sensitization of morphine- and cocaine-induced increase in DA levels in the nucleus accumbens (171). Since 18-MC is a specific blocker of $\alpha 3^*\beta 4^*$ nACRs (171), the possible role of this block as the main mechanism of action of 18-MC was studied. Two lines of evidence pointed to $\alpha 3^*\beta 4^*$ nACRs as the primary target for 18-MC. First, combinations of sub-effective doses of 18-MC and other $\alpha 3\beta 4^*$ nACRs antagonist such as mecamylamine or bupropion also decreased morphine, methamphetamine, and nicotine self-administration in rats (171). Second, micro-injection of 18-MC in the MHb or the IPN, two areas dominated by $\beta 4^*$ nACRs, is sufficient to attenuate DA sensitization to morphine in the nucleus accumbens (148). The fact that $\beta 4$ nAChR null mice show no somatic signs of nicotine withdrawal (19) is in agreement with a possible major role of the habenular/interpeduncular system in drug addiction.

Anatomical Connections Between the Habenula and Mesencephalic Dopaminergic Areas. The habenular complex is an epithalamic area composed of both medial and lateral compartments that receive massive afferents from several DA-rich areas (medial frontal cortex, nucleus accumbens, olfactory bulb, septum and striatum) via the stria medullaris thalami. The main efferent pathway is the fasciculus retroflexus, which projects to IPN, VTA, substantia nigra (SN), medial raphe complex, locus coeruleus, and central gray (172–176). These anatomical connections support the notion that dopaminergic transmission in the midbrain might be regulated by inputs traveling along the fasciculus retroflexus and suggest an important role of the habenular complex as a modulatory relay between limbic forebrain structures and the midbrain.

Modulation of Dopaminergic Activity and Relay of Negative Feedback. Animal studies provide evidence that the VTA and SN receive inhibitory input from the habenula. Electrical stimulation of the habenular nuclei causes inhibition of ~85–90% of the DA neurons in the VTA and SN in rats (164). In contrast, habenular lesions increase DA turnover in the NAcc and prefrontal cortex, reflecting an activation of the dopaminergic system (166, 177). The interest in this brain area has been increased by recent data in both human and non-human primates. Experiments conducted in behaving monkeys have shown that the habenula is a source of negative reward signals in DA neurons and that it plays an important role in determining the reward-related activity of DA neurons (163). Those data are corroborated by functional MRI (fMRI) studies in humans showing that when a decision-making error is made and negative feedback is received (when expected reward fails to occur), the habenular nuclei are strongly activated (178, 179). Based on this information, we hypothesize that the activity of the habenula is increased during withdrawal, possibly through the activation of $\beta 4^*$ nAChRs. While the lateral habenula sends direct projections to the midbrain, including the DA neurons in the SN pars compacta and VTA, and the 5-HT neurons in the dorsal raphe (175), the MHb sends most projections to the IPN (180). The IPN in turn sends projections to the raphé nuclei and the VTA (180–182). Therefore, the MHb likely influences monoaminergic transmission via its connections to the IPN.

Expression Patterns of nAChRs in the Habenula and IPN. nAChRs are found on the soma of MHb cells, which express particularly high levels of mRNA for $\beta 4$ and $\alpha 3$ and, to a lesser extent, for $\alpha 5$ (183–186). A combination of whole-cell recording and single cell RT-PCR techniques showed that, at least in the habenular areas studied, 95–100% of MHb cells express $\alpha 3$, $\alpha 4$, $\alpha 5$, $\beta 2$ and $\beta 4$, whereas approximately 40% of the cells express $\alpha 6$, $\alpha 7$ and $\beta 3$ (187). Recent work using $\beta 4$ -selective monoclonal antibodies confirmed prominent immunoreactivity in the MHb (188). The MHb provides dense cholinergic innervation to the IPN with projections that run ipsilaterally in the fasciculus retroflexus (180, 189). The presynaptic terminals that regulate ACh release onto the IPN may also express $\alpha 3^*\beta 4^*$ nAChRs, as shown by experiments using α -conotoxin AulB and $\beta 2$ null mutants (190). IPN neurons express nAChR complexes containing both $\beta 2$ and $\beta 4$ (191, 192) along with $\alpha 2$. Based on the literature, most MHb neurons express $\alpha 3^*\beta 4^*$ nAChRs, while in the IPN $\alpha 2$ would be the most likely partner for $\beta 4$, possibly in combination with $\alpha 5$.

Concluding Remarks

The nicotinic system and its roles in physiology and disease, including addiction, are actively being studied by different techniques. One of the most prolific approaches has been the study of subunit-specific mutant mice. From an

assessment of the literature, a major role for $\alpha 4^*\beta 2^*$ receptors in reward seems apparent. This effect is mediated by the VTA/NAcc dopaminergic connection. In contrast, nicotine withdrawal, especially the somatic signs, seem to be mediated by $\beta 4^*$ nAChRs in the MHb and IPN.

To design improved anti-tobacco therapies we must focus on the withdrawal symptoms that appear upon smoking cessation. The wealth of information on the nicotinic system and its roles on tobacco addiction is moving us closer to that goal.

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